EXPERIMENTAL NEUROLOGY 67, 52–78 (1980)

Locus Ceruleus Region: Effects on Behavior of Cholinergic, Noradrenergic, and Opiate Drugs Injected Intracerebrally into Freely Moving Cats

P. A. M. VAN DONGEN[1]

Department of Pharmacology and Department of Anatomy and Embryology, University of Nijmegen, P.O.B. 9101, 6500 HB Nijmegen. The Netherlands.

Received May 14, 1979

Behavioral effects of intracerebral injections of small amounts of cholinergic, noradrenergic, and opiate agents into 125 sites in and around the locus ceruleus of the cat were investigated. Many effects were found that could be related to different regions. Carbachol caused atonia comparable to the atonia during paradoxical sleep; the most effective regions were the nucleus pontis centralis caudalis and the subceruleus region. Carbachol injected into the nucleus pontis centralis oralis caused defense reactions like hissing and growling and disturbed vocalizations. Micturation and defecation were elicited by carbachol injected near the nucleus laterodorsalis tegmenti. Carbachol caused ipsiversive and contraversive circling when injected in or near the region of the norepinephrine-containing cell bodies; this circling was probably not due to the NE cells. α-Noradrenergic and opiate agonists injected into the part of the nucleus pontis centralis oralis just rostral to the locus ceruleus caused behavioral inactivation. α-Noradrenergic agonists caused vomiting; the most effective region was the fourth ventricle. Naloxone injected intracerebrally in morphine-treated cats

Abbreviations: LC—locus ceruleus, SC—subceruleus, PCO—nucleus pontis centralis oralis, PCC—nucleus pontis centralis caudalis, NG—nucleus gigantocellularis, NE—norepinephrine, LdT—nucleus laterodorsalis tegmenti, LCd—locus ceruleus pars dorsalis.

[1] The author wishes to express his appreciation for the constant support extended by Dr. A. R. Cools: Thanks to his encouragement and helpful criticism these investigations and this manuscript are substantially improved. The author also thanks his colleagues in the Departments of Pharmacology and Anatomy for helpful discussion of the results and in the preparation of this manuscript. We thank Mrs. Roelie T. de Boer-van Huizen for her excellent histological assistance and Mrs. Margot Klumpkens for her accurate secretarial assistance. We are indebted for the supply of drugs used in this investigation to Boehringer-Ingelheim (clonidine), Janssen Pharmaceutica (fentanyl), and Endo-Laboratories (naloxone).

0014-4886/80/010052-27$02.00/0

antagonized the morphine-induced stereotyped behavior and part of the behavioral disturbance; it is suggested that this is due to the NE cells: An adequate sensorimotor coordination was dependent on an intact central NE transmission. It is suggested that the locus ceruleus acts as a sympathetic nucleus situated in the brain with extensive parts of the central nervous system as its target regions.

INTRODUCTION

Since the discovery that the locus ceruleus (LC) contains the greatest number of noradrenergic cell bodies in the central nervous system (19, 63) many investigations have been devoted to the anatomy and function of this small nucleus [for review see (3)]. The LC was reported to be involved in paradoxical sleep, waking, nociception, anxiety, reward, extinction, stimulus sampling, regulation of micturation, cardiovascularrenal functions, and reducing the reaction to stress (3, 15, 17, 34, 40, 54). In most studies lesions or electrical stimulation were used, whereas only incidentally the intracerebral injection technique was used (9, 51, 62). The advantages of the intracerebral injection technique are a more selective influence on cell bodies than on passing fibers, and the possibility of influencing a specified population of receptors. A disadvantage is the unpredictable spread of the drug, but this can be met by the use of low doses, small volumes, and a great number of injection sites.

In the present study the effects of cholinergic, (nor)adrenergic, and opiate agonists injected intracerebrally into 125 sites in and around the LC of cats were investigated. These drugs are selected because the activity of the LC cells is increased by acetylcholine (7) and decreased by α-norepinephrine(NE)-agonists, especially clonidine (10) and opiate agonists (7). A systematic exploration of behavioral effects evoked by drugs injected into this region of freely moving cats was undertaken to analyze the behavioral phenomena in which the LC and its surrounding regions are involved. Some effects partially or totally published previously (64, 65) will be mentioned briefly here, because the combined occurrence of all effects is part of the subject of this paper.

METHODS

Animals. In this study 45 adult and young adult cats of either sex were used. From a large stock healthy cats were selected which approached the experimentator purring and rubbing their heads against the experimentator's hand or against objects when their cages were opened ("gentle cats"). The cats were housed individually in stainless-steel cages (60 × 40 × 40 cm) under standard laboratory conditions.

Surgery. Pentobarbital (Nembutal, 30 mg/kg, i.p.) anesthesia was used in 31 cats; if necessary an additional injection (5 to 10 mg/kg, i.p.) of

pentobarbital or thiopental (Nesdonal) was made. The remaining 14 cats received initially ketamine (Vetalar, 20 to 30 mg/kg, i.m.), then anesthesia was continued with halothane (artificial ventilation with N_2O-O_2, 2:1). The halothane concentration was set at a level that caused complete abolishment of the hind limb flexor reflex after a single pinch of the skin between the toes (0.4 to 0.8% of halothane). Double-barrel stainless-steel cannulae were used; the diameter of the outer cannula was 0.8 mm and of the inner (dummy) cannula 0.5mm; its tip extended 1 mm beyond the outer cannula (12). Cannulae directed 2 mm above the region of the LC, (Horsley–Clarke coordinates: P 1.5 to 2.5, L 2.0 to 2.5, H −0.3 to −2.0) were bilaterally implanted. They were placed in a parasagittal plane at an angle of 30° (Fig. 6) piercing the visual cortex, superior or inferior colliculus, and the lateral periaqueductal gray. The day after the operation the general state of the cats was checked and several reflexes were tested.

Equipment. The observation box (80 × 80 × 80 cm) stood in a sound-attenuated room; food, water, and a cat's box were absent. The behavior was observed via closed-circuit television and recorded on tape. The camera was 2.5 m from the observation box; its position and its zoom lens (11.5 to 90 mm) were remotely controlled.

Procedure. Details of the procedure were published elsewhere (66, 67). Two days before the experiments the cats were familiarized with the observation box. When female cats were rolling and adopted the posture for copulation at a touch of their backs, it was added to the protocols that they were in heat. A standard ethogram (11) was used for description of the behavior. Cats received intracerebral injections (usually unilateral, always 0.5 μl) manually with a Hamilton syringe, the needle of which extended 2 mm beyond the site of the tip of the dummy cannula (duration of the injection about 1 s). The behavior after injection was observed during at least 45 min, the first 15 min of which the animals were left undisturbed; only effects beginning in these first 15 min are included in this report. Behavioral testing of animals showing muscular atonia is described elsewhere (66); in three cases electromyograms of the neck muscles were made. The experimental procedure used to study the effects of systemic administration of morphine and intracerebral injections of naloxone are extensively described elsewhere (67). In short, the cats received morphine (5 mg/kg, i.p.) and 40 min thereafter an intracerebral injection of naloxone (0.8 to 10 μg). With the dose and schedule of morphine injections used no signs of morphine dependence were observed; all effects mentioned in this report were observed in naive cats and in cats treated 2 days to 4 weeks earlier with morphine. After some series of experiments, the original dummy cannula was replaced by a cannula 1 to 2 mm longer to test sites situated more ventrocaudally.

Localization. At the end of the experiments the cats were anesthetized with pentobarbital and transcardially perfused with saline followed by Susa solution. The brains were sectioned in a parasagittal plane and stained with Nissl's stain. Of the 125 injection sites used in this study, 113 were histologically localized [cf. (66)].

The demarcation of anatomic regions is according to Taber (64), Berman (6), and Maeda *et al.* (39). The demarcation of the region of the NE cells is indicated in Fig. 1 according to Maeda *et al.* (39) and Poitras and Parent (49). The pontine reticular formation rostral to the stereotaxic P 3.0

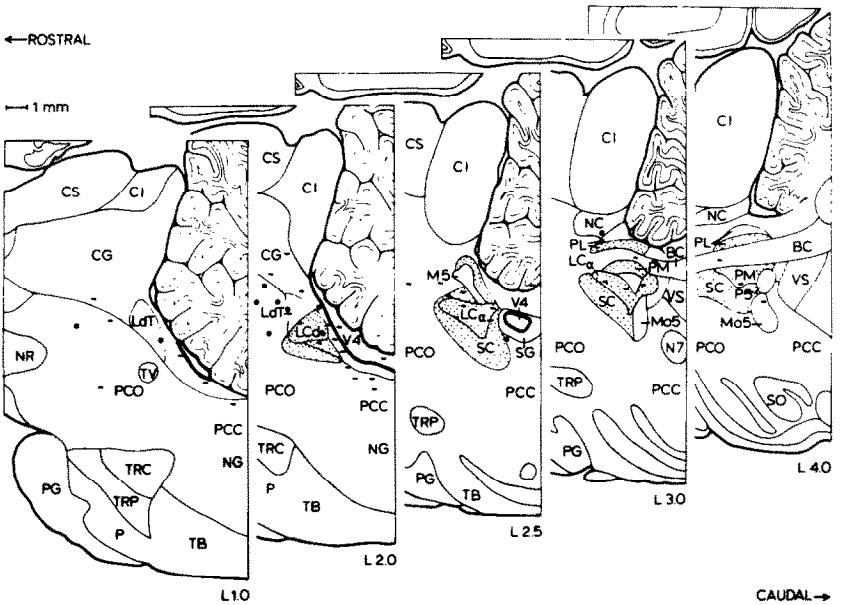

FIG. 1. Localization of the pontine NE cells (stippled) in the pontine tegmentum of the cat. Localization of the injection sites from which clonidine (5 μg) elicited behavioral inactivation. ●—behavioral inactivation, ·—no behavioral inactivation. Abbreviations in this and subsequent figures: BC—brachium conjunctivum, CG—central gray, CI—colliculus inferior, CS—colliculus superior, LCd—locus ceruleus pars dorsalis, LCα—locus ceruleus pars ventralis, LdT—nucleus laterodorsalis tegmenti, M5—nucleus tractus mesencephali nervi trigemini, Mo5—nucleus motorius nervi trigemini, N7—nervus facialis, NC—nucleus cuneiformis, NG—nucleus gigantocellularis, NR—nucleus ruber, P—tractus pyramidalis, P5—nucleus sensorius superior nervi trigemini, PCC—nuclei pontis centralis caudalis, PCO—nucleus pontis centralis oralis, PG—pontine gray (nuclei pontis), PL—nucleus parabrachialis lateralis, PM—nucleus parabrachialis medialis, SC—subceruleus region, SO—oliva superior, TRC—nucleus reticularis tegmenti pontis, pars centralis, TRP—nucleus reticularis tegmenti pontis, pars pericentralis, TV—nucleus tegmenti ventralis, VL—nucleus vestibularis lateralis, and VS—nucleus vestibularis superior.

plane is called nucleus pontis centralis oralis (PCO), and the region caudal to this plane nucleus pontis centralis caudalis (PCC). The region called PCC in this study, according to Taber (64), is the rostral part of the region designated gigantocellular tegmental field by Berman (6) and Amatruda *et al.* (4). The localization of the injection sites is indicated in composite drawings.

Data Analysis. The regions with most of the effective sites with regard to each separate effect were demarcated. Four distinct regions were statistically analyzed: the NE region, the central gray, the PCO, and the PCC; inside each of these regions at least 10 injection sites were present. For each effect a 2×4 contingency table (effective–ineffective sites for four regions) was made and a χ^2 test was used to indicate the region–effect relationship. Then, a 2×2 contingency table (inside–outside versus effective–ineffective) was made for each of the four regions, and the probability (P) for finding the observed number of effective sites by chance was calculated: $P = 4P'$, where P' was calculated following the two-tailed accurate binominal approximation (43) (the factor 4 is a correction for the number of tests). For each effect the number of effective sites in and near each region (i.e., at a distance of less than 1 mm) was determined. To test the combined occurrence of effects, a correlation matrix was made; the probability of combined occurrences was again calculated according to the two-tailed accurate binominal approximation (43).

Drugs. The following drugs were used (the numbers between the brackets indicate the dose and the number of injection sites tested with the drug): carbamylcholine–HCl (carbachol, Sigma, 0.05 to 0.5 μg, $N = 125$), atropine–H_2SO_4 (Ned. Farm., 1 μg, $N = 8$), mecamylamine–HCl (Merck, Sharp and Dohme, 0.6 μg, $N = 6$), *l*-norepinephrine–HCl (Fluka, 10 μg, N = 13), clonidine–HCl (Boehringer-Ingelheim, 5 μg, $N = 47$), oxymetazoline–HCl (Ciba Arnhem, 5 μg, $N = 9$), isoprenalinebitartrate (Sigma, 5 μg, $N = 9$), piperoxane–HCl (Brocades, 5 μg, $N = 9$), yohimbine–HCl (Nogepha, $N = 3$), *l*-propranolol–HCl (ICI, 10 μg, $N = 10$), *d*-amphetamine–H_2SO_4 (Brocades, 5 μg, $N = 4$), desipramine–HCl (Pertofran, Geigy, 5 μg, $N = 4$), apomorphine–HCl ("De Onderlinge Pharmaceutische Groothandel" OPG, 5 μg, $N = 3$), morphine–HCl (OPG, 5 μg, $N = 12$), fentanyl-citrate (Janssen, 5 μg, $N = 13$), naloxone–HCl (Endo Laboratories, 0.8 to 10 μg, $N = 48$), procaine–HCl (OPG, 5 μg, $N = 23$), L-glutamic acid (Boom Meppel, 5 μg, $N = 11$), and saline (0.5 μl, $N = 45$). All drugs were dissolved in saline, except yohimbine (distilled water) and morphine (saline or distilled water). All doses refer to the salts.

RESULTS

The differential behavioral effects will be presented separately. The injection sites were scattered in and around the NE region (Figs. 1, 3–5, 7). The amount of tissue destruction due to the injections was small (Fig. 6). A more extensive lesion of brain tissue was noted around the injection site in one animal, so the data of this animal were discarded.

The effects of repeated injections of the same dose of a drug into the same site in the dorsolateral pontine tegmentum were tested with an intertrial interval of 2 to 7 days. An initial effective site was also effective at the second trial in 81% of the cases (Table 1). An initial effective injection site could become ineffective; in these cases no tissue destruction nor granulation tissue could be detected in the Nissl-stained material that could explain the loss of effectiveness.

Muscular Atonia

Behavior Description. The cholinergic agonist carbachol (0.5 μg, unilateral) caused muscle relaxation (42 effective sites) [for an extensive description see (66)]. This was accompanied by a loss of tonus of the neck muscles (Fig. 2). The 25 effective sites from a previous study were included in this report. The carbachol-induced atonia was reproducible in 79% of the experiments ($N = 19$): three previously effective sites became ineffective and four previously ineffective sites became effective (Table 1).

Other Drugs. The other drugs did not mimic the carbachol-induced atonia (see Methods for dose and number of injection sites). The following

TABLE 1

Effects of Repeated Injections in the Dorsolateral Pontine Tegmentum[a]

	+ +	+ −	− +	− −	Reproducibly effective (%)
Carbachol-induced atonia	15	4	3	22	79
Carbachol-induced asymmetry	17	5	2	20	77
Clonidine-induced vomiting	10	2	0	10	83
Naloxone-induced antagonism	5	0	0	6	100
Total	47	11	5	58	81

[a] + +, both injections effective; + −, first injection effective, second injection ineffective; − +, first injection ineffective, second injection effective; − −, both injections ineffective.

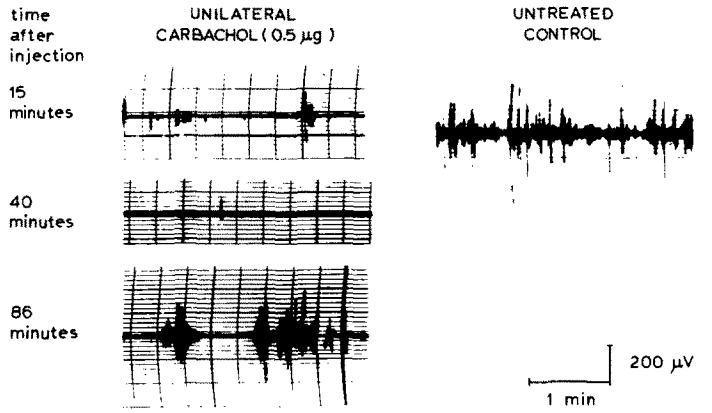

FIG. 2. Electromyogram of the muscles of the neck of a cat after a unilateral intracerebral injection of carbachol; note the absence of muscle tone.

drugs were tested in at least two carbachol-effective sites: *l*-NE, clonidine, oxymetazoline, isoprenaline, piperoxane, *l*-propranolol, desipramine, apomorphine, morphine, fentanyl, naloxone, procaine, and L-glutamic acid.

Localization. The region from which carbachol (0.5 μg) induced atonia was situated ventral to the LC (H −3.0), and caudal to the level of the tegmental nuclei of Gudden (P 1.0) [see also (66)]. It extended at least from L 1.0 to L 4.0, but the ventral and caudal borderlines of the effective region could not be determined from the available injection sites. The effective region included the subceruleus region (SC), the caudal part of the PCO, the PCC, and the nucleus gigantocellularis (NG). The region–effect relationship tended to be significant ($\chi^2 = 6.76$, df = 3, $0.05 < P < 0.10$). The most effective regions were the PCC (percentage effective sites: in/near 46%, $N = 37$; outside 21%, $N = 76$) and the SC region (percentage effective sites: in/near 38%, $N = 56$; outside 21%, $N = 57$) (Table 2).

Asymmetric Behavior

Behavior Description. Asymmetric behavior was elicited by unilateral injections of carbachol (0.5 μg). An injection was considered to elicit asymmetric behavior when the cat held its head turned sideward at an angle of more than 90° with the body axis for more than 1 min during the first 15 min after the injection; this could be accompanied by rotations. When the cat turned during grooming, it was not counted as asymmetric behavior. According to this criterium, asymmetric behavior occurred after a unilateral injection of carbachol (0.5 μg) in 40 of 125 cases: 10 ipsiversive and 30 contraversive. Injections of saline into this region never caused

TABLE 2

Distribution of Effective Injection Sites for Anatomically Demarcated Regions[a]

		Ventr.		CG		LC		SC		NE		LdT		PCO		PCC		
			+1		+1		+1		+1		+1		+1		+1		+1	Total
Carbachol-induced:																		
Atonia	+	0	2	2	9	3	8	5	21	8	21	0	3	10	17	12	17	33
	−	2	16	11	32	12	29	10	35	24	47	3	17	20	37	12	20	80
Asymmetry																		
ipsi		0	0	0	1	2	4	1	5	3	7	0	3	4	6	0	0	7
no		2	10	6	24	8	17	9	34	17	39	1	8	23	38	17	28	77
contra		0	8	7	16	5	16	5	17	12	22	2	9	3	10	7	9	29
Growling/hissing	+	0	0	1	2	0	0	1	6	1	6	0	1	9	10	0	2	11
	−	2	18	12	39	15	37	14	50	31	62	3	19	21	44	24	35	102
Vocalizations	+	0	2	1	4	1	4	0	3	1	5	1	4	7	8	2	2	12
	−	2	16	12	37	14	33	15	53	31	63	2	16	23	46	22	35	101
Elimination	+	0	4	4	7	2	7	0	6	3	8	0	6	2	6	2	5	10
	−	2	14	9	34	13	30	15	50	29	60	3	14	28	48	22	32	103
Total carbachol		2	18	13	41	15	37	15	56	32	68	3	20	30	54	24	37	113
Clonidine-induced:																		
Vomiting	+	2	9	5	7	0	4	0	6	0	6	0	1	1	4	2	5	10
	−	0	7	7	22	9	15	3	12	14	20	2	11	11	18	4	6	37
Inactivation	+	0	4	1	8	1	5	0	3	1	7	2	5	5	7	1	1	11
	−	2	12	11	21	8	14	3	15	13	19	0	7	7	15	5	10	36
Total clonidine		2	16	12	29	9	19	3	18	14	26	2	12	12	22	6	11	47
Naloxone-induced:																		
Normalization	+	0	3	2	7	3	7	1	11	4	12	0	1	2	6	6	8	14
	−	0	7	7	19	6	12	1	16	10	19	3	11	9	18	7	9	34
Total naloxone		0	10	9	26	9	19	2	27	14	31	3	12	11	24	13	17	48

[a] For abbreviations see Fig. 1.

asymmetric behavior. The mean latency of asymmetric behavior was 2 min, 5 s (range 30 s to 5 min); the duration was more than 45 min in all experiments. Asymmetric behavior was reproducible in 77% of the tests ($N = 22$).

In 20 of the 125 cases the cats made six rotations or more during the first 15 min, which is the minimum to reach $P < 0.05$ in a two-tailed binomial test. Of these 20 cases three made statistically significant more rotations to the ipsilateral side and nine to the contralateral side. In cases of ipsiversive and contraversive rotation the cats were circling about stationary hind limbs. The turning was compulsive: when the animals were raised from the floor, they still tried to turn in circles. Contraversive turning and a statistically significant number of contralateral rotations occurred after unilateral injections of 50 ng carbachol ($N = 3$). The asymmetric behavior caused by 0.5 μg carbachol was antagonized by intracerebral injections of the muscarine receptor antagonist atropine (1 μg, $N = 3$) and not by an equivalent dose of the nicotine receptor antagonist mecamylamine (0.6 μg, $N = 2$). Antagonism of ipsiversive rotations was not tested.

Other Drugs. The other drugs did not mimic the carbachol-induced

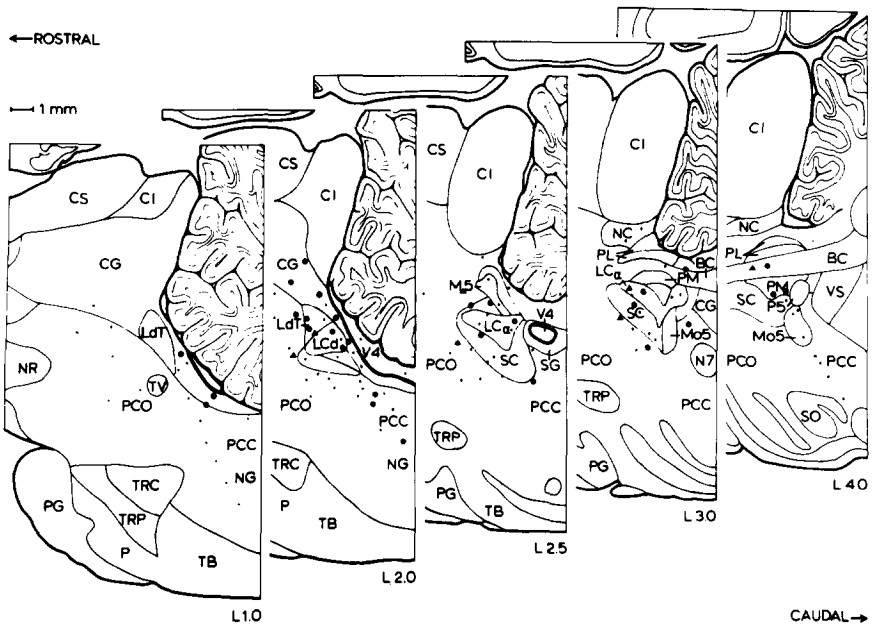

FIG. 3. Localization of the injection sites from which a unilateral intracerebral injection of carbachol (0.5 μg) elicited turning. ●—contraversive turning, ▲—ipsiversive turning, •—no turning. For the localization of the NE cells, see Fig. 1.

asymmetric behavior (see Methods for dose and number of sites). The following drugs were tested in at least two carbachol-effective sites: *l*-NE, clonidine, oxymetazoline, isoprenaline, piperoxane, *l*-propranolol, *d*-amphetamine, morphine, fentanyl, procaine, and L-glutamic acid.

Localization. For the carbachol-induced asymmetric behavior a region–effect relationship was present ($\chi^2 = 15.61$, df = 6, $0.01 < P < 0.02$). *Ipsiversive* turning was elicited exclusively from injection sites in the rostral pole of the NE region, and the PCO just rostral of it, i.e., near the nucleus laterodorsalis tegmenti (LdT) (Fig. 3). Most effective were the NE region (percentage effective sites: in/near 15%, $N = 68$; outside 0%, $N = 45$) and the LdT (percentage effective sites: in/near 15%, $N = 20$; outside 4%, $N = 93$). Ineffective was the PCC (percentage effective sites: in/near 0%, $N = 37$; outside 13%, $N = 76$). Sites in the fourth ventricle were ineffective. Sites from which *contraversive* turning was elicited were situated in a plane at an angle of 45° with the horizontal plane from rostrodorsal to ventrocaudal partly along the ventricular wall (Fig. 3). From the sites available the dorsal and ventral limits of this plane could not be determined. Effective regions were in and near the caudal central gray (CG) (percentage effective sites: in/near 40%, $N = 41$; outside 20%, $N = 72$) and the NE region (percentage effective sites: in/near 36%, $N = 68$; outside 18%, $N = 45$) (Table 2). Sites in the fourth ventricle were ineffective.

Growling and Hissing

Behavior description. Growling and/or hissing was noticed after unilateral injections of carbachol (0.5 μg) in 11 of 125 sites. These sites were found in seven cats (three males and four females). Growling and hissing occurred 15 to 210 min after the injection, when the cats were approached by the experimentator. It was accompanied by open-mouth threats and attempts to hit with the claws and to bite, followed by quick withdrawal. When handled, these cats bit or hit the experimentator suddenly. Other elements of the agonistic behavior occurred: small pupils ($N = 3$), wide pupils ($N = 6$), sideward-turned ears ($N = 3$), flattened ears ($N = 6$), and lashing tail movements ($N = 11$). (Lashing tail movements and small or wide pupils were noted after injections into more sites, but only sites where growling and hissing were elicited were regarded as effective.) Other vocalizations from the agonistic repertoire, namely caterwauls of fighting tomcats, or screams did not occur. In 2 out of these 11 cases spontaneous hissing was observed: without any noticeable change in the observation room these cats suddenly turned their back to the rearwall, hissed, and raised their forepaws. The latencies of this behavior were 6 min, 15 s and 14 min, 20 s.

At two of the three sites tested, the growling and hissing was reproducible. (In one animal four repeated injections of carbachol caused growling, hissing, and biting, after which the experiments with this animal had to be terminated because it could not be handled without severe constraint.) The days before and after the carbachol experiments the other cats that directed their defense reaction to the experimentator reacted with purring and rubbing their heads on objects when the experimentator approached them, as they did formerly. The cats that were growling and hissing did not show signs of distress before or during the injection. A dose of 50 ng carbachol was not effective ($N = 3$) at sites where one of 0.5 μg was.

Other Drugs. The other drugs did not mimic the carbachol-induced growling and hissing (see Methods for dose and number of sites). The following drugs were tested in at least two carbachol-effective sites: clonidine, fentanyl, procaine, and *L*-glutamic acid.

Localization. For the carbachol-induced growling and hissing a region–effect relationship was present ($\chi^2 = 81.36$, df = 3, $P < 0.001$). The most effective area was the PCO ($P = 8 \times 10^{-4}$, Fig. 4) (percentage

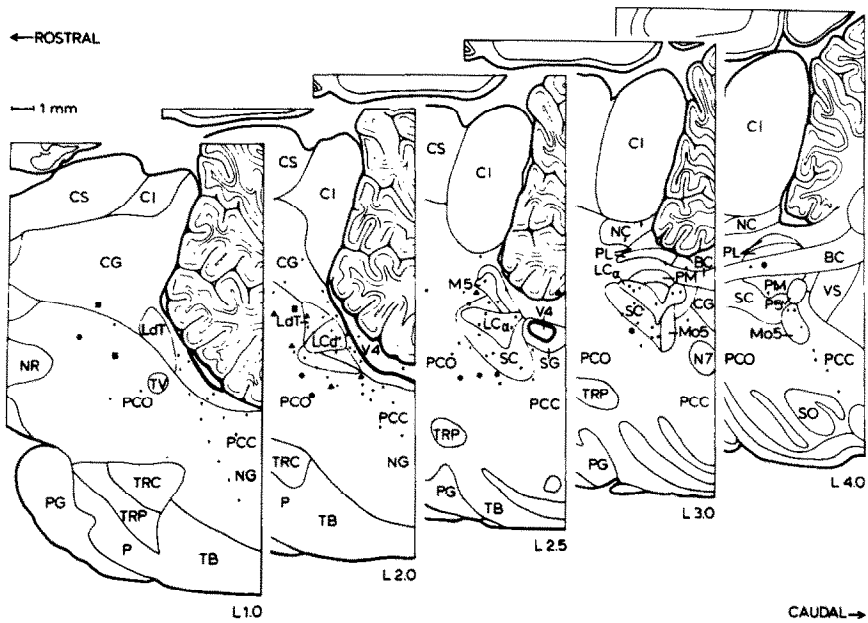

FIG. 4. Localization of the injection sites from which a unilateral injection of carbachol (0.5 μg) elicited growling and hissing, or elongated vocalizations. ●—growling and hissing, ▲—elongated vocalizations, ■—both growling and hissing and elongated vocalizations. For the localization of the NE cells, see Fig. 1.

effective sites: in/near 19%, $N = 54$; outside 2%, $N = 59$). No effective sites were present in the PCC nor in the fourth ventricle (Table 2).

Elongated Vocalizations

Behavior Description. Elongated, hoarse vocalizations were heard after unilateral injections of carbachol (0.5 μg) into 12 of the 125 sites in 11 cats (eight males and three females; none of the females was in heat). The vocalizations were similar in different cats. Normal mews had a duration of 0.2 to 0.6 s, incidentally to 1.0 s. The mean duration of the elongated vocalizations was 4.8 $\pm$ 0.2 s (mean and SE, range 1.2 to 10.6 s, 265 consecutive vocalizations in six cats). The mean latency was 5 min (range 2 min, 5 s to 10 min, 15 s) and the mean duration 8 min, 45 s (range 1 min, 15 s to 18 min, 30 s). The elongated vocalizations were emitted in bursts of 6 to 13 vocalizations per minute, alternated with single vocalizations for some time. Such vocalizations were never heard from untreated cats: They were different from the elongated, high pitched cries of kittens left alone, from the elongated caterwauls of fighting tomcats, and from the elongated cries of female cats in heat.

Other Drugs. Eight sites where carbachol caused elongated vocalizations were tested with procaine (5 μg); at four of these sites, procaine induced similar vocalizations (mean latency 45 s, mean duration 2 min, 20 s). At 15 sites where carbachol did not elicit elongated vocalizations, procaine was also ineffective. The other drugs did not mimick the carbachol-induced, elongated, hoarse vocalizations (see Methods for dose and number of sites). The following drugs were tested in at least two carbachol-effective sites: clonidine, oxymetazoline, fentanyl, and L-glutamic acid. Incidentally, injections of saline and some other drugs (oxymetazoline 5 μg, piperoxane 2 $\times$ 5 μg, morphine 2 $\times$ 5 μg) were followed by an increase in the frequency of vocalizations, but these were normal with respect to pitch and duration, and they were elicited from sites other than those where carbachol caused elongated, hoarse vocalizations.

Localization. For the carbachol-induced elongated vocalizations the region–effect relationship tended to be significant ($\chi^2 = 6.94$, df = 3, $0.05 < P < 0.10$). Most effective sites were found in and near the PCO (Fig. 4) (percentage effective sites: in/near 15%, $N = 54$; outside 7%, $N = 59$; Table 2). Effective sites were not found more lateral than the L 2.0 plane nor in the fourth ventricle.

Elimination (Defecation and Micturation)

Behavior Description. Micturation and defecation was observed after unilateral intracerebral injections of carbachol (0.5 μg) in 10 of the 125

sites. The way the cats micturated or defecated was species-specific; sniffing of the feces or urine and digging movements occurred. Some animals adopted only the posture of defecation (flexion in the hip and knee, and extension in the heel) and made movements with the flanks without elimination. Cats remaining 2 h in a familiar observation box without a cat's box did not eliminate. Micturation and defecation are indicated separately in Fig. 5, but are treated in the further analysis as a single effect, elimination, because both effects could be elicited from identical or adjacent sites. The mean latency of elimination was 4 min, 30 s (range 1 min, 15 s to 8 min, 55 s). Elimination was reproducible ($N = 3$) and caused by as little as 50 ng carbachol ($N = 2$).

Other Drugs. At sites where carbachol did not cause elimination, a bilateral injection of fentanyl (2 × 5 μg) caused defecation in 3 of 13 cases, but unilateral injections of fentanyl (5 μg) at one of those sites were ineffective. At three carbachol-ineffective sites clonidine (5 μg) caused micturation; at these sites clonidine also caused vomiting (see below); they were situated in and near the fourth ventricle. The other drugs did not cause

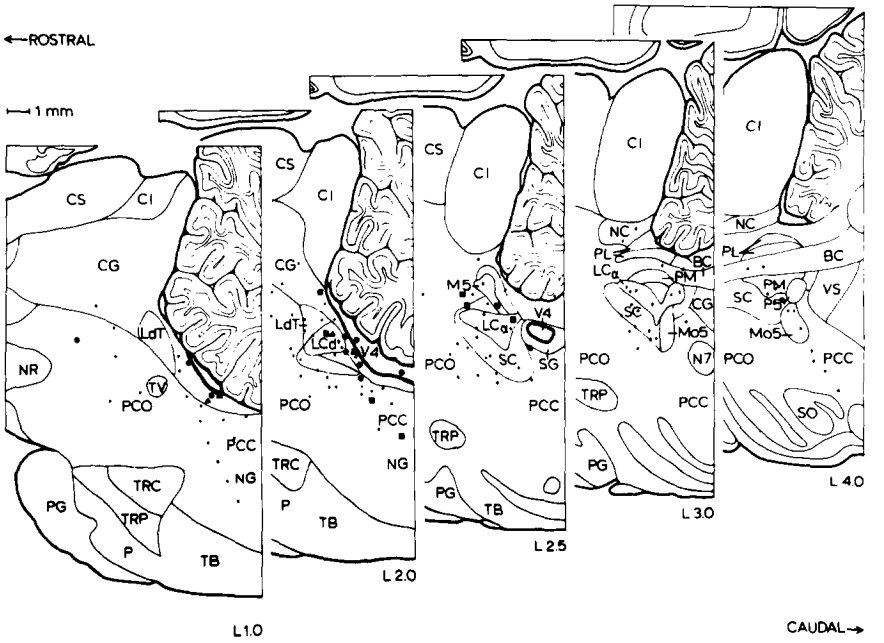

FIG. 5. Localization of the injection sites from which unilateral injections of clonidine (5 μg) elicited vomiting, and from which unilateral injections of carbachol (0.5 μg) elicited defecation and micturation. ●—clonidine-induced vomiting, ■—carbachol-induced defecation, ▲—carbachol-induced micturation. For the localization of NE cells, see Fig. 1.

defecation or micturation. The following drugs were tested in at least two carbachol-effective sites: clonidine, oxymetazoline, and fentanyl.

Localization. For carbachol-induced elimination the region–effect relationship tended to be significant ($\chi^2 = 7.35$, df = 3, $0.05 < P < 0.10$). The effective region was situated in a plane with an angle of 45° with the horizontal plane, along the ventricular wall from at least L 1.0 to L 2.5 (Fig. 5); it partly overlapped with the region from which the contraversive rotations were elicited. The most effective region was near the LdT (Fig. 5) (percentage effective sites: in/near 30%; $N = 20$; outside 4%, $N = 93$) and the central gray ($P = 0.0068$) (percentage effective sites: in/near 17%, $N = 41$; outside 4%, $N = 72$). Sites in the fourth ventricle were not effective.

Vomiting

Behavior Description. After a unilateral injection of the α-noradrenergic agonist clonidine (5 μg), vomiting was observed in 10 of the 47 cases. This vomiting was similar to normal vomiting. Initially, the cats rhythmically put out their tongues, and opened and closed their mouths swallowing for 1 to 2 min. Thereafter they vomited with rhythmical movements of the flanks and the head, making some steps backward. Sniffing the vomit occurred regularly, and licking and eating it were observed occasionally. A single injection caused this pattern two to five times, but after two to three times the animals only retched. The clonidine-induced vomiting was reproducible in 83% of the cases ($N = 12$). The mean latency of the first vomiting was 6 min, 20 s (range 4 min to 9 min, 25 s). One microgram clonidine did not induce vomiting ($N = 5$), when injected at sites where 5 μg was effective.

Characterization of the Receptors. At clonidine-effective sites the α-noradrenergic receptor agonist oxymetazoline (5 μg, $N = 5$) was also effective, whereas the β-noradrenergic receptor agonist isoprenaline (5 μg, $N = 5$) was ineffective. The clonidine-induced vomiting was antagonized by α-noradrenergic receptor antagonists; yohimbine (0.5 mg/kg, i.p., 30 min before clonidine, $N = 3$) prevented vomiting, and piperoxane (10 μg, intracerebral, at the same site as clonidine and administered just before clonidine, $N = 2$) attenuated the vomiting.

Other Drugs. At clonidine–effective sites the clonidine–induced vomiting was not mimicked by *d*-amphetamine (5 μg, $N = 4$), desipramine (5 μg, $N = 4$), morphine (5 μg, $N = 5$), fentanyl (5 μg, $N = 5$), or apomorphine (5 μg, $N = 3$), although the last three drugs caused vomiting when administered systemically. At 2 of the 10 clonidine–effective sites, carbachol (0.5 μg) caused retching and was in-

effective at the remaining 8 clonidine–effective sites. After intracerebral injections of the other drugs mentioned under Methods, at sites where clonidine did not elicit vomiting, no vomiting or retching was observed. At 3 of the 10 sites where clonidine caused vomiting, clonidine also caused micturation. At other sites clonidine never caused micturation. The association between the clonidine–induced micturation and the clonidine–induced vomiting was statistically significant ($P = 0.015$).

Localization. For the clonidine–induced vomiting a region–effect relationship was present ($\chi^2 = 8.50$, df = 3, $0.02 < P < 0.05$). Evidently, injection sites in or near the fourth ventricle were most effective (Fig. 5) (percentage of effective sites: in/near 56%, $N = 16$; outside 3%, $N = 31$) (Table 2). Also in the central gray many effective sites were found, but these were in connection with the fourth ventricle: either the shaft of the cannula had opened the ventricular wall or granulation tissue was formed between the cannula track and the ventricular wall (Fig. 6). Clonidine elicited vomiting only in one site remote from the ventricle. No effective sites were in the NE region.

Activity

Behavior Description. After unilateral intracerebral injections of clonidine (5 μg in 11 of 47 sites) and after bilateral injections of morphine (2×5 μg; in 6 of 12 cats) the cats were behaviorally sedated, sitting with their eyes closed and hardly moving. A trained observator of cat behavior judged it as abnormal drug-induced inactivation. After a bilateral injection of fentanyl (2×5 μg) cats were placed in an unfamiliar observation box; 4 of 13 cats interrupted their exploration behavior and became inactive (mean latency 4 min, 30 s, range 3 min to 6 min, 40 s; mean duration 6 min, 15 s, range 3 min to 11 min). When the cats were placed in an unfamiliar observation box, the time spent walking and sniffing was decreased to 50 and 30%, respectively (6 to 7 min after injection) for the whole group ($N = 18$), compared with the saline-treated controls. In the cats where fentanyl (bilateral) caused an interruption of the exploration behavior, clonidine unilaterally injected either at the right or at the left side caused inactivation.

Other Drugs. Unilateral injections of carbachol (0.5 μg) only at 2 of the 125 sites tested caused a similar inactivation; at both sites clonidine was ineffective. Of course, the cats showing carbachol–induced atonia were inactive, but clonidine-, morphine-, or fentanyl-treated cats were not atonic and clonidine did not cause inactivation at sites where carbachol caused atonia (cf. Table 3).

Localization. The sites from which clonidine induced inactivation were

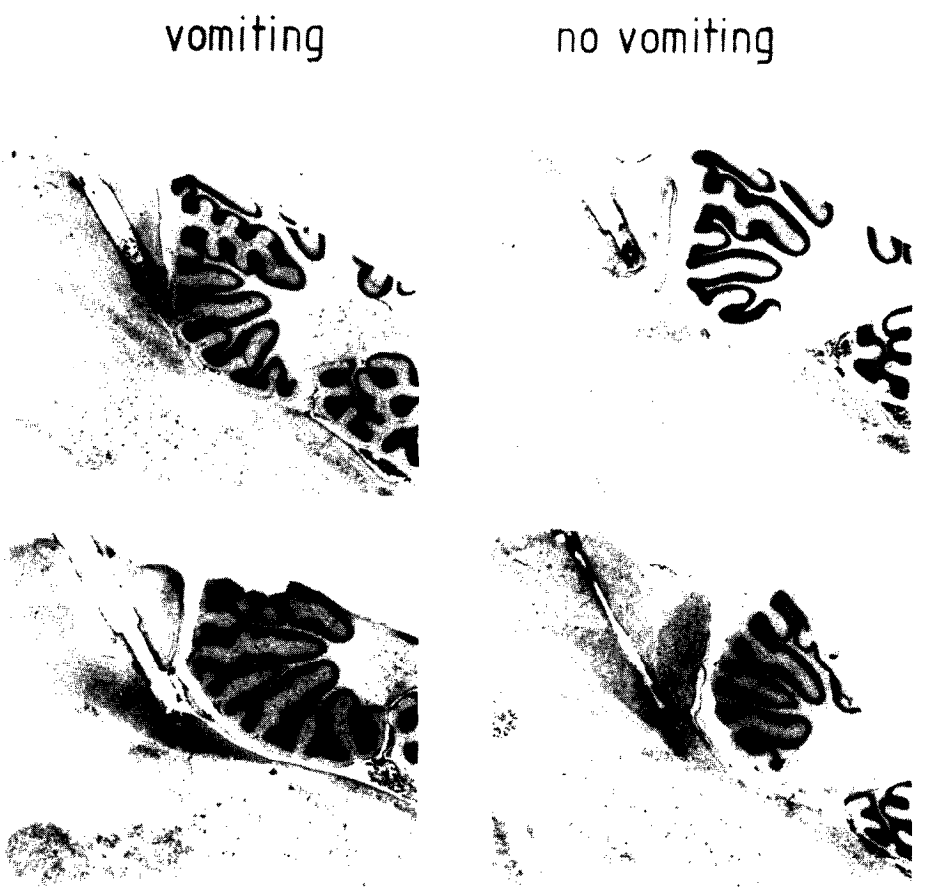

FIG. 6. Nissl-stained parasagittal sections showing the tracks of the cannulae near the ventricle from which clonidine did or did not elicit vomiting.

clustered rostrodorsal to the LC pars dorsalis (LCd) and the Ldt (Fig. 1). Most effective sites were in and near the LdT (percentage effective sites: in/near 42%, $N = 12$; outside 6%, $N = 35$) and the PCO (percentage effective sites: in/near 32%, $N = 22$; outside 4%, $N = 25$) (Table 2). Sites in the fourth ventricle were not effective. Due to the bilateral injections no relationship between morphine- or fentanyl-induced effects and injection sites could be settled.

Naloxone-Induced Normalization of Morphine-induced Behavior

Behavior Description. The effects mentioned below were more extensively described previously (67). Morphine-treated (5 mg/kg, i.p.)

TABLE 3

Correlation Matrix for the Combined Occurrence of Different Drug-Induced Effects from Identical Injection Sites[a]

	Carbachol-induced						Clonidine-induced	
	Asymmetry contra	Asymmetry ipsi	Atonia	Growling/hissing	Vocalization	Elimination	Vomiting	Inactivation
Carbachol-induced (N = 125)								
Atonia	−0.36	0.26						
Growling/hissing	−0.04	−0.11	−0.04					
Vocalization	0.16	−0.09	−0.16	0.22				
Elimination	0.46	−0.04	−0.14	−0.10	0.01			
Clonidine-induced (N = 47)								
Vomiting	−0.13	−0.19	−0.07	0.08	−0.20	−0.01		
Inactivation	0.16	−0.15	−0.23	0.27	0.39	−0.19	−0.16	
Naloxone-induced (N = 48)								
Normalization	0.14	−0.15	0.08	−0.07	0.08	0.20	−0.14	0.26

[a] Indicated is the ϕ coefficient. P values according to Moolenaar (43).

cats showed repetitive sequences of disintegrated patterns of poorly coordinated behavior (13) and reacted inadequately to their environment (68). After a bilateral (2 × 0.8 μg, N = 15) or a unilateral (2 μg, N = 48) injection of the morphine receptor antagonist, naloxone, into the dorsolateral pontine tegmentum the cats could interrupt the repeated performance of the sequences of disintegrated behavior, and instead of the morphine–induced disturbance of behavior they showed an improved sensorimotor coordination (mean latency 1 min, 15 s, range 30 s to 7 min; mean duration 5 min, range 2 min, 35 s to 8 min). This effect was reproducible (Table 1) and dose-dependent.

Localization. Most sites from which naloxone caused partial antagonism of morphine–induced behavior were located in and near the NE region (Fig. 7) (percentage effective sites: in/near 39%, N = 31; outside 12%, N = 17) (Table 2), and also many effective sites were found in the PCC near the NE region (percentage effective sites: in/near 47%, N = 17; outside 19%, N = 31) (Table 2). Few effective sites were found in and near the LdT and none in the fourth ventricle.

Combined Effects

A correlation matrix of the different effects was made (Table 3). This table should be interpreted with caution because the effects observed might be a priori dependent. For example, a combination of carbachol-induced

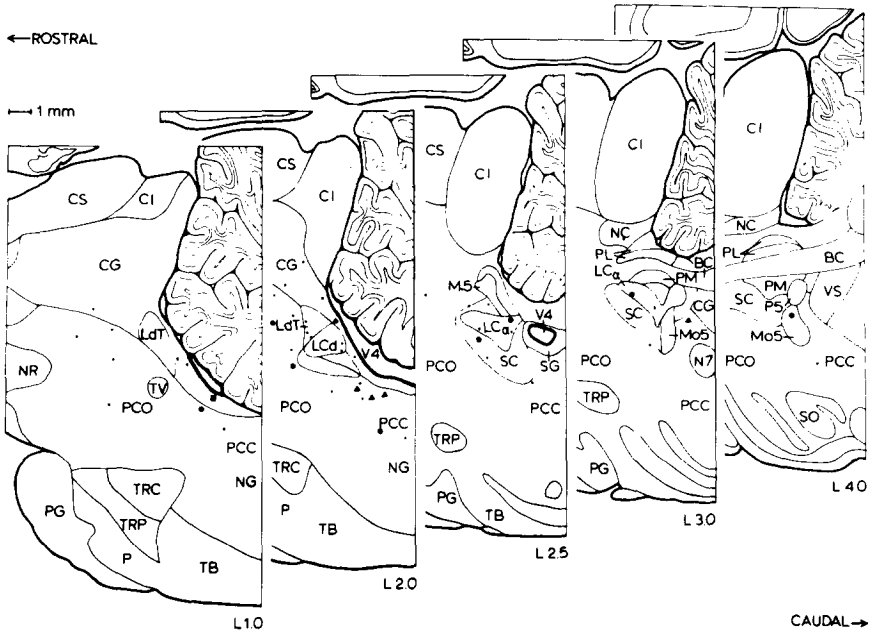

FIG. 7. Localization of the injection sites from which naloxone (2 μg) elicited partial antagonism of morphine-induced behavior. ●—antagonism of morphine-induced head movements, ▲—antagonism of morphine-induced stereotyped patterns, ■—antagonism of morphine-induced stereotyped patterns and active normalized behavior, •—no naloxone-induced effects. For the localization of the NE cells, see Fig. 1.

atonia and carbachol-induced asymmetric behavior might be impossible; for ipsiversive turning and carbachol-induced atonia, however, a positive association was found ($P = 0.028$), so the strong negative association between carbachol-induced atonia and carbachol-induced contraversive turning ($P < 0.001$) was not an a priori result. Effects elicited from about the same region were always positively associated: carbachol-induced hissing/growling and carbachol-induced elongated vocalization and clonidine-induced inactivation (all in PCO), carbachol-induced contraversive turning and carbachol-induced elimination (both in/near the central gray), and clonidine-induced inactivation and naloxone-induced normalization of behavior (both in/near the NE region). A positive association was found between the clonidine-induced vomiting and the clonidine-induced micturation ($P = 0.015$; not in Table 3). The combined occurrence of clonidine-induced vomiting and all other effects shown in Table 3 were tested: the expected combined occurrences are tested against the observed combined occurrences: A negative association exists ($P < 0.05$, Wilcoxon).

DISCUSSION

The Intracerebral Injection Technique

The various techniques to manipulate brain regions have their specific advantages and disadvantages (44, 45, 52, 56, 59). The advantage of the intracerebral injection technique is the possibility of studying acute effects, to affect specific receptors, and to exclude effects due to interactions of the drugs with passing fibers, when local anesthetic effects are excluded. The major disadvantage of the intracerebral injection technique is the unpredictable spread of the drug, which can be restricted or extensive (44, 45, 56). The amount of drug which can be found in the target region, fluctuates considerably among different injection sites [cf. tables in (44, 45)]. So, injections in the effective region can be ineffective, even when the dose is well above the "real" threshold dose. Moreover, small to considerable amounts of the drugs can be found back in remote regions (44, 45, 56); this can cause the occurrence of effective sites outside the effective region. These limitations seem to be intrinsic to the intracerebral injection technique. When a large number of injection sites in combination with low doses of drugs is used, these limitations are overcome. This appeared an effective strategy to ascribe drug-induced effects to limited anatomic regions.

Another problem has to be solved: cholinergic and adrenergic drugs not only influence neurons but also the local cerebral circulation. β-Adrenoceptor-stimulating agents and low doses of cholinergic agonists cause vasodilatation and α-adrenoceptor-stimulating agents and high doses of cholinergic agonists cause vasoconstriction (22). Yet, neither α- nor β-adrenoceptor-stimulating agents (clonidine, oxymetazoline, isoprenaline) nor *l*-NE mimicked the effects of the cholinergic agonist carbachol. So, the effects were probably not due to their influence on the local cerebral circulation.

Effects via the Ventricle? Clonidine-Induced vomiting

α-Noradrenergic receptor agonists (clonidine, oxymetazoline) injected into the fourth ventricle caused vomiting or retching. Similar effects occur after systemic injections of clonidine or other centrally acting sympathomimetic drugs (23, 50). Comparison of the threshold doses after systemic injections with those of intracerebral injections (20 μg/kg and 5 μg, respectively) makes it unlikely that the vomiting after intracerebral injections was due to diffusion of the drug to the peripheral system. Yohimbine antagonized the vomiting induced by clonidine systemically (23) or intracerebrally administered; so the receptors involved are α-NE-like receptors.

Injections into and near the fourth ventricle were most effective (Fig. 6, Table 2). Because only sites in the central gray which were in connection with the fourth ventricle were effective, it can be safely assumed that the vomiting-inducing α-NE-like receptors are not situated in the central gray, but are elsewhere in or near the ventricular wall. The more remote area postrema in the ventricular wall is reported to be involved in vomiting (8), and in this region (nor)-adrenergic (dopamine-β-hydroxylase-containing) terminals are found in close relation with the ependymal cells of the ventricular wall (65); their activity is probably mimicked by injection of α-adrenoceptor agonists in the fourth ventricle. Clonidine caused vomiting from only one site remote from the ventricle; this indicated that, as a rule, only a pharmacologically insignificant amount of clonidine reached the ventricle. The negative association between sites from which clonidine-induced vomiting was elicited and sites from which the other effects were elicited, indicates that none of the other effects was mediated via the ventricle.

Carbachol-Induced Atonia

With regard to the carbachol-induced atonia, the present study corroborates conclusions of an earlier study in which a smaller number of injection sites was used (66). In this study evidence is presented that the muscles are indeed atonic. The occurrence of effective sites was high in the SC region and in the PCC. Because (i) effective sites were found medial, ventral, and caudal to the NE region in this and in other studies (4, 14), and (ii) the carbachol-induced atonia is not influenced by α- and β-NE-receptor blocking agents, nor by an intracerebral injection of the catecholamine-selective neurotoxin 6-hydroxydopamine (66), we may conclude that the carbachol-induced atonia is mediated via the pontine reticular formation and not via the NE cells. As no effective sites were found in the PCO rostral to the stereotaxic P 1.0 plane, the PCC rather than the PCO mediates the carbachol-induced atonia.

Because the carbachol-induced atonia is related to atonia during paradoxical sleep (4, 42), our data might imply that the PCC, and not the pontine NE cells, generates atonia during paradoxical sleep. This is in agreement with recently reported conclusions that (i) the presence of LC cells, or of NE at terminal sites is not necessary for paradoxical sleep [cf. (32)] and (ii) LC cells are silent during paradoxical sleep (25, 33). Furthermore, the descending NE fibers of the LC and SC to the spinal motor regions (47) enable locomotion (28) rather than generate atonia.

Carbachol-Induced Asymmetric Behavior

Asymmetric behavior was elicited by carbachol probably via muscarinic receptors because atropine but not mecamylamine blocked these

carbachol-induced effects. From many sites in and near the NE region ipsiversive as well as contraversive turnings could be elicited (Fig. 3). Experiments with electrical stimulation (17) and lesions of the NE region (20, 21) yielded either ipsiversive, contraversive, or no rotations. Because (i) clonidine, which decreases the activity of the LC cells (10), did not induce rotation (ii) the NE cells project bilaterally (31), and (iii) electrolytic lesions produce stronger rotation than the more specific 6-hydroxydopamine-induced lesions (21), we suggest that the NE cells do not significantly contribute to turning behavior.

Manipulations in the dorsolateral pontine tegmentum yielded ipsiversive as well as contraversive rotations. This again underlines that rotations can not be simply used as a specific model for one transmitter system.

Defense Behavior

The agonistic behavior after a number of injections of carbachol (growling, hissing, attempts to hit and bite) was similar to the species-specific defense reaction of the cat (38). Evidently, the effective region in this respect was the PCO. This is in agreement with other studies with carbachol injections or electrical stimulation of this region or the adjacent mesencephalic central gray (1, 5, 30). The carbachol-induced defense reaction cannot be regarded as an emotional reaction to the carbachol-induced atonia, because both effects were not correlated (Table 3) and were elicited from different regions.

Electrical stimulation of the NE region is also reported to yield reactions like those to threatening stimuli in the monkey (53, 54), and feeling of fear in man [(46), precise localization is lacking]; in the rat, however, flight reactions are observed only very rarely (17, 61). On the contrary, lesions of the LC or its ascending fibers increase behavioral measures of anxiety (18, 41). Because (i) in our study defense reactions were elicited from the PCO, and (ii) the threshold stimulation current is generally lower for myelinated fibers than for cell bodies (52), our data and the data of Redmond (54) are in agreement, if one assumes that the flight and defense reactions caused by electrical stimulation were due to stimulation of afferent or efferent fibers of the PCO.

Elongated Vocalizations

Carbachol injected into the PCO caused hoarse vocalizations with a longer duration than normal mews. These vocalizations can be ascribed to actions on cell bodies as well as on passing fibers, because procaine injected into this region caused similar vocalizations. The elongated vocalizations cannot be regarded as an emotional reaction to the carbachol-induced atonia, because both effects were not correlated and were

elicited from different regions. Electrical stimulation in this region also yielded vocalization, sometimes associated with growling (35, 36). Both defense reactions and elongated vocalization were elicited from the PCO, but the structures mediating both effects must be different, because (i) procaine never caused defense reactions, and (ii) both effects also occurred separately as well after injections of carbachol as after electrical stimulation (36).

Elimination

Carbachol often caused defecation and micturation when injected near the LdT; this is in agreement with findings of Hernández-Péon *et al.* (30). Although electrical stimulation and lesions of the NE region were reported to have urogenital effects (2, 27, 32, 55), recent functional and anatomic findings indicate that these effects are mediated via the LdT rather than via the NE cells (58). Although effects on defecation after lesions or electrical stimulation of the LC region were described (32, 57), our data indicate that these effects are due to influences on afferent or efferent fibers of the LdT rather than to influences on the NE cells.

Activity

The activity of the cats was diminished both after a unilateral injection of clonidine just rostral to and into the LCd, and after bilateral injections of morphine and fentanyl. This is in agreement with the finding that morphine injected near the LC of rats also decreased activity measured in self-stimulation experiments and in the open field situation [(9) and personal communication]. However, there are some conflicting data in this respect (62).

Both NE agonists (especially clonidine) and opiate agonists (morphine and fentanyl) suppress the maintained activity of LC cells (7, 10, 37), and the opiate receptor binding sites and opiate-sensitive cells were reported to be abundant in the LC and rare in the surrounding regions (7, 48). So, an attractive interpretation might be to impute behavioral effects elicited both by NE agonists and by opiate agonists injected into a region close to the LC to diffusion of the drugs to the LC region. But we do not share this interpretation for the clonidine-induced inactivation, because effective injection sites were not clustered around but were just rostral to the LC. Moreover, most authors do not find a change in the activity of rats after destruction of the LC or its ascending fibers [e.g., (18, 55)], but a few authors describe a decrease in the activity (2). Our data in the cat together with the above investigations in the rat suggest that a decrease in the activity of the animals is due to the portion of the PCO just rostral to the NE cells.

Partial Antagonism of Morphine-induced Behavior

Previously we presented evidence in favor of the hypothesis that the naloxone-induced partial antagonism of morphine-disturbed behavior was due to the action of naloxone on the NE cells (67). Morphine systemically administered decreased the activity of the LC cells (37), disturbed the reaction of cats to their environment (68), and caused stereotyped behavior (13). Naloxone antagonized the morphine-induced decrease in the activity of the LC cells (7). Thus, naloxone injected into and near the NE region of morphine-treated cats can be assumed to restore the activity of the NE cells, and it diminished the disturbance in the sensorimotor coordination in these animals and the occurrence of morphine-induced stereotyped movements. Accordingly, we put forward the hypothesis that (i) morphine-induced decrease in the activity of the pontine NE cells is a necessary condition for the occurrence of the morphine-induced sensorimotor disturbance, and (ii) naloxone, inducing restoration of the activity of the NE cells of morphine-treated cats, is able to restore the sensorimotor and integrative functions of the parts of the central nervous system that receive terminals from the NE region.

General

As mentioned above, restoration of the activity of the NE cells of morphine-treated cats is suggested to restore the reactions of the animal to its environment. This is in agreement with the following findings: (i) activity of the LC cells increases the signal-to-noise ratio of cerebellar Purkinje cells (26), hippocampal pyramidal cells (60), and auditory cortex cells (24); and (ii) the LC is suggested to be involved in filtering away irrelevant stimuli (40).

Morphological resemblance exists between the pontine NE cells and the peripheral (sympathetic) NE cells (3, 29). Electrical stimulation of the LC increases the turnover of peripheral NE via the sympathetic system (16). These considerations give support to the hypothesis of Hartman and Udenfriend (29) that there is also a functional resemblance: the central NE cells prepare the central nervous system to cope with a crisis situation.

REFERENCES

1. ALLIKMETS, L. H. 1974. Cholinergic mechanisms in aggressive behaviour. *Med. Biol.* **52**: 19–30.
2. AMARAL, D. G., AND J. A. FOSS. 1975. Locus ceruleus lesions and learning. *Science* **188**: 377–378.
3. AMARAL, D. G., AND H. M. SINNAMON. 1977. The locus ceruleus: neurobiology of a central noradrenergic nucleus. *Prog. Neurobiol.* **9**: 147–196.
4. AMATRUDA, T. T., III, D. A. BLACK, T. M. MCKENNA, R. W. MCCARLY, AND J. A.

HOBSON. 1975. Sleep cycle control and cholinergic mechanisms: differential effects of carbachol injections at pontine brain stem sites. *Brain Res.* **98:** 501–515.

5. BAXTER, B. L. 1968. Elicitation of emotional behavior by electrical or chemical stimulation applied at the same loci in cat mesencephalon. *Exp. Neurol.* **21:** 1–11.
6. BERMAN, A. L. 1968. *The Brain Stem of Cat. A Cytoarchitectonic Atlas with Stereotaxic Coordinates.* Univ. of Wisconsin Press, Milwaukee.
7. BIRD, S. J., AND M. J. KUHAR. 1977. Iontophoretic application of opiates to the locus ceruleus. *Brain Res.* **122:** 523–533.
8. BORISON, H. C. 1977. Area postrema: chemoreceptor trigger zone for vomiting—Is that all?. *Life Sci.* **14:** 1807–1817.
9. BROEKKAMP, C. L. E., J. H. VAN DEN BOGAARD, H. J. HEIJNEN, R. H. ROPS, A. R. COOLS, AND J. M. VAN ROSSUM. 1976. Separation of inhibiting and stimulating effects of morphine on self-stimulation behaviour by intracerebral microinjections. *Eur. J. Pharmacol.* **36:** 443–446.
10. CEDARBAUM, J. M., AND G. K. AGHAJANIAN. 1977. Catecholamine receptors on locus ceruleus neurons: pharmacological characterizations. *Eur. J. Pharmacol.* **44:** 375–385.
11. COOLS, A. R., AND J. M. VAN ROSSUM. 1970. Caudate dopamine and stereotyped behavior of cats. *Arch. Int. Phramacodyn.* **187:** 163–173.
12. COOLS, A. R. 1971. The function of dopamine and its antagonism in the caudate nucleus of cats in relation to the stereotyped behaviour. *Arch. Int. Pharmacodyn.* **194:** 259–269.
13. COOLS, A. R., H. J. JANSSEN, AND C. L. E. BROEKKAMP. 1974. The differential role of the caudate nucleus and the linear raphe nucleus in the initiation and the maintenance of morphine-induced behaviour in cats. *Arch. Int. Pharmacodyn.* **210:** 163–174.
14. CORDEAU, J. P., A. MOREAU, A. BEAULNES, AND C. LAURIN. 1963. EEG and behavioral changes following microinjections of acetylcholine and adrenaline in the brain stem of cats. *Arch. Ital. Biol.* **101:** 30–47.
15. CORRODI, H., K. FUXE, P. LIDBRINK, AND L. OLSON. 1971. Minor tranquilizers, stress, and central catecholamine neurons. *Brain Res.* **29:** 1–16.
16. CRAWLEY, J. N., S. T. HATTOX, J. W. MAAS, AND R. H. ROTH. 1978. 3-Methoxy-4-hydroxyphenethylene glycol increase in plasma after stimulation of the nucleus locus ceruleus. *Brain Res.* **141:** 380–384.
17. CROW, T. J., P. J. SPEAR, AND G. W. ARBUTHNOT. 1972. Intracranial self-stimulation with electrodes in the region of the locus ceruleus. *Brain Res.* **36:** 275–287.
18. CROW, T. J., J. F. W. DEAKIN, S. E. FILE, A. LONGDEN, AND S. WENDLANDT. 1978. The locus ceruleus noradrenergic system—evidence against a role in attentuation, habituation, anxiety and motor activity. *Brain Res.* **155:** 249–261.
19. DAHLSTRÖM, A., AND K. FUXE. 1964. Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. *Acta Physiol. Scand.* (Suppl.) 232.
20. DONALDSON, I. MACG., A. DOLPHIN, P. JENNER, C. D. MARSDEN, AND C. PYCOCK. 1976. The roles of noreadrenaline and dopamine in contraversive circling behaviour seen after unilateral electrolytic lesions of the locus ceruleus. *Eur. J. Pharmacol.* **39:** 179–191.
21. DONALDSON, I. MACG., A. DOLPHIN, P. JENNER, C. D. MARSDEN, AND C. PYCOCK. 1976. The involvement of noradrenaline in motor activity as shown by rotational behaviour after unilateral lesions of the locus ceruleus. *Brain.* **99:** 427–446.
22. EDVINSSON, L. 1975. Neurogenic mechanisms in the cerebro vascular bed. Autonomic nerves, amine receptors and their effects on cerebral blood flow. *Acta Physiol. Scand. (Suppl.)* 427.

23. FLORIO, V., L. BIANCHI, AND V. G. LONGO. 1975. A study of the central effects of sympathomimetic drugs: EEG and behavioural investigations on clonidine and naphazoline. *Neuropharmacology.* **14:** 707–714.
24. FOOTE, S. L., R. FREEDMAN, AND A. P. OLIVER. 1975. Effects of putative neurotransmitters on neuronal activity in monkey auditory cortex. *Brain Res.* **86:** 229–242.
25. FOOTE, S., AND F. E. BLOOM. 1979. Activity of locus ceruleus neurons in the unanaesthetized squirrel monkey. Pages 625–627 in E. USDIN, Ed., *Catecholamines: Basic and Clinical Frontiers.* Pergamon, New York.
26. FREEDMAN, R., B. J. HOFFER, D. J. WOODWARD, AND D. PURO. 1977. Interaction of norepinephrine with cerebellar activity evoked by mossy and climbing fibers. *Exp. Neurol.* **55:** 269–288.
27. GEORGE, R., W. L. HASLETT, AND P. LOMAX. 1962. Hematuria following midbrain lesions in the rat. *Exp. Neurol.* **6:** 349–356.
28. GRILLNER, S. 1975. Locomotion in vertebrates: central mechanisms and reflex interaction. *Physiol Rev.* **55:** 247–304.
29. HARTMAN, B. K., AND S. UDENFRIEND. 1972. The application of immunological techniques to the study of enzymes regulating catecholamine synthesis and degradation. *Pharmacol. Rev.* **24:** 311–330.
30. HERNÁNDEZ-PEÓN, R., G. CHAVEZ-IBARRA, P. MORGANE, AND C. TIMO-IARIA. 1963. Limbic cholinergic pathways involved in sleep, and emotional behavior. *Exp. Neurol.* **8:** 93–111.
31. JONES, B. E., AND R. Y. MOORE. 1977. Ascending projections of the locus ceruleus in the rat. II. Autoradiographic study. *Brain Res.* **127:** 23–53.
32. JONES, B. E., S. T. HARPER, AND A. E. HALARIS. 1977. Effects of locus ceruleus lesions upon cerebral monoamine content, sleep-wakefulness states and the response to amphetamine in the cat. *Brain Res.* **124:** 473–496.
33. JONES, G., M. SEGAL, S. FOOTE, AND F. E. BLOOM. 1979. Locus ceruleus neurons in freely moving rats exhibit pronounced alterations of discharge rate during sensory stimulation and stages of the sleep-wake cycle. Pages 643–645 in E. USDIN, Ed., *Catecholamines: Basic and Clinical Frontiers.* Pergamon, New York.
34. Jouvet, M. 1972. The role of monoamines and acetylcholine-containing neurons in the regulation of the sleep-wake cycle. *Ergebn. Physiol.* **64:** 166–307.
35. JÜRGENS, U., AND D. PLOOG. 1970. Cerebral representation of vocalization in the squirrel monkey. *Exp. Brain Res.* **10:** 532–554.
36. KANAI, T., AND S. C. WANG. 1962. Localization of the central vocalization mechanism in the brain stem of the cat. *Exp. Neurol.* **6:** 426–434.
37. KORF, J., B. S. BUNNEY, AND G. K. AGHAJANIAN. 1974. Noradrenergic neurons: morphine inhibition of spontaneous activity. *Eur. J. Pharmacol.* **25:** 165–169.
38. LEYHANSEN, P. 1956. *Verhaltenstudien am Katzen.* Paul Parey Verlag, Berlin.
39. MAEDA, T., C. PIN, D. SALVERT, M. LIGIER, AND M. JOUVET. 1973. Les neurones contenant des catecholamines du tegmentum pontique et leur voies de projection chez le chat. *Brain Res.* **57:** 119–152.
40. MASON, S. T. 1978. Parameters of the dorsal bundle extinction effect: previous extinction experience. *Pharmacol. Biochem. Behav.* **8:** 655–659.
41. MASON, S. T., D. C. S. ROBERTS, AND H. C. FIBIGER. 1978. Noradrenaline and neophobia. *Physiol. Behav.* **21:** 353–361.
42. MITLER, M. M., AND W. C. DEMENT. 1974. Cataplectic-like behavior in cats after micro-injections of carbachol in pontine reticular formation. *Brain Res.* **68:** 335–343.
43. MOLENAAR, W. 1970. *Approximations to the Poisson, Binomial and Hypergeometric Distribution Functions.* Ph.D. Thesis, Mathematical Center, Amsterdam.

44. MYERS, R. D., M. TYTELL, A. KAWA, AND T. RUDY. 1971. Micro-injection of ^{3}H-acetylcholine, ^{14}C-serotonin and ^{3}H-norepinephrine into the hypothalamus of the rat: diffusion into tissue and ventricles. *Physiol. Behav.* **7:** 743–751.
45. MYERS, R. D., AND D. B. HACK. 1978. ^{14}C-Dopamine microinjected into the brainstem of the rat: dispersion kinetics, site content and functional dose. *Brain Res. Bull.* **3:** 601–609.
46. NASHOLD, B. S. JR., W. P. WILSON, AND G. SLAUGHTER. 1974. The midbrain and pain. Pages 191–196 in I. BONICA AND J. JOSEPH, Eds., *Advances in Neurology, Vol. 4*. Raven Press, New York.
47. NYGREN, L.-G., AND L. OLSON. 1977. A new major projection from locus ceruleus: the main source of noradrenergic terminals in the ventral and dorsal columns of the spinal cord. *Brain Res.* **132:** 85–93.
48. PERT, C. B., M. J. KUHAR, AND S. H. SNYDER. 1976. Opiate receptor: autoradiographic localization in rat brain. *Proc. Nat. Acad. Sci. U.S.A.* **73:** 3729–3733.
49. POITRAS, D., AND A. PARENT. 1978. Atlas of the distribution of monoamine-containing nerve cell bodies in the brain stem of the cat. *J. Comp. Neurol.* **179:** 699–718.
50. PUTKONEN, P. T. S., A. LEPPÄVUORI, AND D. STENBERG. 1977. Paradoxical sleep inhibition by central alpha-adrenoceptor stimulant clonidine antagonized by alpha-receptor blocker yohimbine. *Life Sci.* **21:** 1059–1066.
51. RAICHLE, M. E., B. K. HARTMAN, J. O. EICHLING, AND L. G. SHARPE. 1975. Central noradrenergic regulation of cerebral blood flow and vascular permeability. *Proc. Natl. Acad. Sci. U.S.A.* **72:** 3726–3730.
52. RANCK, J. B., JR. 1975. Which elements are excited in electrical stimulation of mammalian central nervous system: a review. *Brain Res.* **98:** 417–440.
53. REDMOND, D. E., JR., Y. H. HUANG, D. R. SNYDER, AND J. W. MAAS. Behavioral effects of stimulation of the nucleus locus ceruleus in the stump-tailed monkey *Macaca arctoidesi. Brain Res.* **116:** 502–510.
54. REDMOND, D. E., JR. 1977. Alterations in the function of the nucleus locus ceruleus: a possible model for studies of anxiety. Pages 293–306 in I. HANIN AND E. USDIN, Eds., *Animal Models in Psychiatry and Neurology*. Pergamon, New York/Oxford.
55. ROBERTS, D. C. S., M. T. C. PRICE, AND H. C. FIBIGER. 1976. The dorsal tegmental noradrenergic projection: an analysis of its role in maze learning. *J. Comp. Physiol. Psychol.* **90:** 363–372.
56. ROUTTENBERG, A. 1972. Intracranial chemical injection and behaviour: a critical review. *Behav. Biol.* **7:** 601–641.
57. RUSSELL, G. V. 1955. The nucleus locus ceruleus (dorsolateralis tegmenti). *Tex. Rep. Biol. Med.* **13:** 939–988.
58. SATOH, K., N. SHIMIZU, M. TOHYAMA, AND T. MAEDA. 1978. Localization of the micturation reflex center at dorsolateral pontine tegmentum of the rat. *Neurosci. Lett.* **8:** 27–33.
59. SCHOENFELD, T. A., AND L. W. HAMILTON. 1977. Secondary brain changes following lesions: a new paradigm for lesion experimentation. *Physiol. Behav.* **18:** 951–967.
60. SEGAL, M., AND F. E. BLOOM. 1976. The action of norepinephrine in the rat hippocampus. IV. The effects of locus ceruleus stimulation on evoked hippocampal unit activity. *Brain Res.* **107:** 513–525.
61. SIMON, H., M. LEMOAL, AND B. CARDA. 1975. Self-stimulation in the dorsal pontine tegmentum in the rat. *Behav. Biol.* **13:** 339–347.
62. SMEE, M. L., P. F. WESTON, D. S. KINNER, AND T. DAY. 1975. Dose-related effects of central noradrenaline stimulation on behavioral arousal in rats. *Psychopharmacol. Comm.* **1:** 123–130.

63. SWANSON, L. W., AND B. K. HARTMAN. 1975. The central adrenergic system. An immunofluorescence study of the location of cell bodies and their efferent connections in the rat utilizing dopamine-β-hydroxylase as a marker. *J. Comp. Neurol.* **163:** 467–505.
64. TABER, E. 1961. The cytoarchitecture of the brain stem of the cat. I. Brain stem nuclei of cat. *J. Comp. Neurol.* **116:** 27–69.
65. TORACK, R. M., P. STRANAKAN, AND B. K. HARTMAN. 1973. The role of norepinephrine in the function of the area postrema. I. Immunofluorescent localization of dopamine-beta-hydroxylase and electron microscopy. *Brain Res.* **61:** 235–252.
66. VAN DONGEN, P. A. M., C. L. E. BROEKKAMP, AND A. R. COOLS. 1978. Atonia after carbachol microinjections near the locus ceruleus in cats. *Pharmacol. Biochem. Behav.* **8:** 527–532.
67. VAN DONGEN, P. A. M., C. L. E. BROEKKAMP, AND A. R. COOLS. 1979. Locus ceruleus and substantia nigra: involvement in morphine-induced behavior. *Brain Res. Bull.*, **4,** 307–311.
68. WIKLER, A. 1944. Studies on the action of morphine on the central nervous system of cat. *J. Pharmacol. Exp. Ther.* **80:** 176–187.